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Beyond circulating microRNA biomarkers: Urinary microRNAs in ovarian and breast cancer

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Abstract

Breast cancer is the most common malignancy in women worldwide, and ovarian cancer is the most lethal gynecological malignancy. Women carrying a BRCA1/2 mutation have a very high lifetime risk of developing breast and ovarian cancer. The only effective risk-reducing strategy in BRCA-mutated women is a prophylactic surgery with bilateral mastectomy and bilateral salpingo-oophorectomy. However, many women are reluctant to undergo these prophylactic surgeries due to a consequent mutilated body perception, unfulfilled family planning, and precocious menopause. In these patients, an effective screening strategy is available only for breast cancer, but it only consists in close radiological exams with a significant burden for the health system and a significant distress to the patients. No biomarkers have been shown to effectively detect breast and ovarian cancer at an early stage. MicroRNAs (miRNAs) are key regulatory molecules operating in a post-transcriptional regulation of gene expression. Aberrant expression of miRNAs has been documented in several pathological conditions, including solid tumors, suggesting their involvement in tumorigenesis. miRNAs can be detected in blood and urine and could be used as biomarkers in solid tumors. Encouraging results are emerging in gynecological malignancy as well, and suggest a different pattern of expression of miRNAs in biological fluids of breast and ovarian cancer patients as compared to healthy control. Aim of this study is to highlight the role of the urinary miRNAs which are specifically associated with cancer and to investigate their role in early diagnosis and in determining the prognosis in breast and ovarian cancer.

Keywords

BRCA, breast cancer, biomarkers, circulating microRNA, urinary microRNA, ovarian cancer

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Introduction

Nowadays, breast cancer (BC) is the most common malignancy in women worldwide and ovarian cancer (OC) is the most lethal gynecological malignancy. The recent evidences emerged in tumor behavior and the dramatic improvements achieved in the treatment of these malignancies, with particular attention in tumor biology and immunobiology,[1–8] less traumatic and more aggressive surgeries,[9–12] and new target drugs,[13–21] have already been highlighted by the same authors elsewhere. Despite all the advances achieved in knowledge and in clinical practice, stage at diagnosis still represents the most important

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prognostic factor and only few patients who are diagnosed with an advanced stage disease can be healed. BC related mortality has dropped significantly since the widespread adoption of mammographic screening.[22] Unfortunately, a similar effective screening methodology that enables an early diagnosis in OC is still lacking.

Approximately 20%–25% of the patients with OC and 5%–10% of the patients with BC carry an inherited predisposition to their pathologic condition.[23] The most commonly involved mutated genes are BRCA 1 and 2. Women carrying a BRCA 1 or 2 mutation (BRCAm) have a 57% and 49% lifetime risk of developing BC and a 40% and 18% risk of developing OC, respectively.[24] Furthermore, once a BRCA 1/2 mutated woman is diagnosed with BC, she has an increased risk of developing a second BC in the contralateral breast. For these patients, the most effective risk-reducing strategy is a prophylactic surgery. Prophylactic bilateral mastectomy and salpingo-oophorectomy have been shown to decrease the incidence of BC and OC in high-risk patients by as much as 90% and 80%, respectively.[25] However, many patients are reluctant to undergo these prophylactic surgeries secondary to the negative impact on the self-image perception derived from the mastectomy and secondary to the precocious menopause, and the loss of fertility, derived from the bilateral salpingo-oophorectomy. Furthermore, nowadays genetic testing is only approved for women with a diagnosis of, or documented familiarity, for BC and OC.

Whereas an increased radiological and clinical surveillance helps in detecting BC at an earlier stage in high-risk patients, no effective screening strategy exists to screen BRCA 1/2 mutated women for OC, once the BRCA mutation has been assessed.

Despite the encouraging role of human epididymis protein 4 (HE4) and HE4 in combination with Carbohydrate-antigen 125 (CA-125) in identifying OC recurrence,[26] these biological markers perform poorly as a screening tool in patients without adnexal masses and cannot be used as diagnostic markers for primary disease due to their low specificity.

New markers are required to identify BC and OC at an early stage when they are highly curable, particularly in patients at high risk of developing these malignancies.

MicroRNAs (miRNAs) are key regulatory molecules operating in the post-transcriptional regulation of gene expression. Aberrant expression of miRNAs has been documented in several pathological conditions, including solid tumors suggesting their involvement in carcinogenesis.

MiRNAs were first discovered in 1993 in the nematode *Caenorhabditis elegans*; they are highly conserved across a wide range of species and have a central role in gene expression by incorporating the RNA-induced silencing complex and interacting with their target messenger RNAs (mRNAs).[27] They comprise approximately 18–22 nucleotides and their regulatory function includes inducing translation suppression or degradation of RNA. One miRNA can bind to several target genes and can be involved in the regulation of various cellular processes such as cell development, differentiation, and proliferation.[28] Since the miRNA loci often map to fragile chromosomal regions interfering with DNA functions (such as, amplifications, deletions, and translocations), their expression is frequently upregulated/downregulated during carcinogenesis.[29,30]

The latest miRNA database (v20, June 2013) contains 24,521 microRNA loci from 206 species, processed to produce 30,424 mature microRNA products.[31] The miRNAs regulate about 30% of all protein-coding genes of the human genome. This can occur via a perfect complimentary binding of the miRNA to the target mRNA (endonucleolytic cleavage of the mRNA) or by an imperfect complimentary binding to the target mRNA (translation repression).[32]

Due to their cell cycle interference, miRNAs are involved either as oncogenes or as oncosuppressors in the pathogenesis of a huge variety of human cancers such as lung cancer,[33,34] prostate cancer,[35] colorectal cancer,[36,37] leukemia,[38–40] gliomas[41] and medulloblastoma,[42] diffuse large B-cell lymphoma,[43] hepatocellular carcinoma (HCC),[44] gastric cancer,[45,46] osteosarcoma,[47] renal cell carcinoma,[48] BC,[49] and OC.[50] Particularly, their presence in the blood has been shown to be associated with histology, clinical stage, survival, and oncogenic expression in OC and BC. Recently, studies have documented the feasibility to detect stable miRNAs in urine samples as well. A direct correlation between miRNAs expression levels in the blood and in the urine has not yet been clearly demonstrated. It is believed that specific metabolic processes in the kidney and in the urothelial tissue can modify the pattern of presentation of miRNAs thus expanding these discrepancies. The occurrence of high levels of RNases in the urinary tract can lead to the total degradation of free RNA types. As a result, only exosomal miRNAs remain detectable in the urine.[51]

Four significantly altered and specifically regulated miRNAs (miR-21, miR-125b, miR-451, and miR-155) were identified in BC patients as compared to healthy controls in a study that evaluated urinary miRNAs expression.[52] These data suggest their potential role as non-invasive innovative biomarkers.

In OC, two important studies have investigated the role of urinary miRNAs.[53,54] Preliminary results show that miRNAs may be significantly upregulated and some exosomal fractions of miRNAs/cell-free miRNAs may be detected in the urine samples of OC patients (miR-21, miR-125b, miR-451, and miR-155).

We aim to give an overview on the studies that investigate the role of urinary miRNAs that are specifically associated with a condition of BC and OC and to give an insight into their diagnostic and prognostic potential.

Rationale and feasibility of miRNAs detection in urine sample

Weber et al.[55] confirmed the presence of miRNAs in 12 human body fluids (plasma, saliva, tears, urine, amniotic fluid, colostrum, breast milk, bronchial lavage,

cerebrospinal fluid, peritoneal fluid, pleural fluid, and seminal fluid).

Urine is the ideal bio-fluid for the biomarker detection as it allows for non-invasive collection. MiRNAs detection in the urine is usually performed either by isolating and extracting total RNA from extracellular vesicles which can be present in the urine samples and by isolating total RNA from the cellular fraction. Briefly, once RNA has been isolated from the urine, small RNA molecules (<200 nt) are amplified, miRNA–complementary DNA (cDNA) probes are diluted in RNAse-free water for subsequent quantitative real-time polymerase chain reaction (qRT-PCR) analysis.

The low number of detectable miRNA species in the urine suggests that the majority of circulating miRNAs is either "picked up" by the kidneys through an unknown mechanism or is destroyed in the urine. Yun et al. validated the stability of miRNAs in the supernatant of the urine. Even after seven cycles of freezing and thawing or a 72 h long storage at room temperature, miRNA levels in the urine remained unchanged.[56]

Generally, urine samples contain lower levels of proteins than blood-based samples, thus reducing protein interference during RNA isolation. However, in the kidney, there is a large amount of nucleases, including RNases, which could lead to the degradation of long-chain RNAs that are unstable in these conditions. In contrast to RNAs, miRNAs are more resistant to nuclease degradation mainly because of their smaller size.

Quantity and quality of urinary miRNAs are the basic features that could influence further analyses. Nowadays, there are no standardized criteria for quality assessment of RNA purified from blood or other body fluids, creating a lack of solid data assessing the application of metabolic signature in urinary samples for the detection of systemic disease.[57]

Urinary miRNAs in non-oncologic conditions: a brief state of the art

Cardiovascular disease

Recently, the diagnostic role of miRNAs has been successfully explored in several settings, such as cardiovascular disease.[58] A systematic search of published original research until 2016 yielded a total of 72 studies, investigating the potential use of miRNAs as diagnostic and/or prognostic biomarkers in plasma and/or serum in patients with atherosclerosis, coronary artery disease, and acute coronary syndrome, and overall 52 different miRNAs were reported as effective. The investigation of miRNAs in the urine of patients affected by acute myocardial infarction reported interesting results.[59] Historically, no good biomarkers are identified in urine after acute myocardial infarction, because the blood protein biomarkers creatinine phosphokinase–muscle band (CPK-MB), troponin T (TnT), and troponin I (TnI) which are currently used as biomarkers for acute myocardial infarction are difficult to be filtered in the urine. The authors showed that urine miR-1 was significantly increased in patients with acute myocardial infarction compared to age and sex-matched healthy controls ($p < 0.05$) and a positive correlation was demonstrated between serum TnT and urine miR-1 levels ($r = 0.70$, $p < 0.05$).

Rheumatology

Recent studies showed that miRNAs play an important role in the regulation of the immune system and in the pathogenesis of autoimmune diseases. The role of miR-155 has been extensively studied in the immune system.[60–62] Mice lacking miR-155 are viable and fertile but are deficient in lymphocyte development and generation of B- and T-cell responses after B-cell receptor or T-cell receptor activation. Also, dendritic cells in miR-155-deficient mice have been shown to have an impaired antigen-presenting function[63] supporting the importance of miR-155 in the immune cells. In men, it has been demonstrated that patients with systemic lupus erythematosus express lower serum miR-146a ($p < 0.05$) and miR-155 levels and higher urinary level of miR-146a ($p < 0.05$). Estimated glomerular filtration rate correlates with both the serum miR-146a ($r = 0.519$, $p = 0.001$) and miR-155 ($r = 0.384$, $p = 0.014$).[64]

Kidney injuries

Unlike liver-specific expression of some miRNA (e.g. miR-122), there are no renal-specific miRNAs. However the uptake from the blood stream by the renal proximal tubular epithelial cells allows for a targeted delivery to the kidney. The renal damage (nephropathy) from diabetes is currently diagnosed and monitored by urinary microalbuminuria. However, microalbuminuria is not specific to diabetic nephropathy. Furthermore, tissue damage and inflammation may have already occurred at the time of detectable microalbuminuria. Biopsy is the present diagnostic and prognostic gold standard test despite its invasiveness and cost. In this scenario, the use of urinary miRNAs as disease biomarkers provides the additional advantages of a new non-invasive testing. Argyropoulos et al.[65] identified a panel of 27 differentially regulated urinary miRNAs that varied with diabetic nephropathy progression. Differential urinary miRNA expression profiles have also been studied in other kidney diseases, such as renal fibrosis and immunoglobulin A (IgA) nephropaty[66] and acute kidney injury.[67] MiR-21, the miR-29 family, and miR-93 have shown to be downstream mediators of the transforming growth factor-1 (TGF-1) in patients with IgA nephropathy. Particularly, the urinary miR-93 level significantly correlated with glomerular scarring ($r = -0.392$,

$p=0.010$) and glomerular filtration rate significantly correlated with urinary levels of miR-21 ($r=0.338$, $p=0.028$), miR-29b ($r=0.333$, $p=0.031$), and miR-29c ($r=0.304$, $p=0.050$.[66]

Dermatology

A panel of miRNAs was identified to be overexpressed in cells from skin lesions from patients affected by atopic dermatitis.[68] Also, miR-203 is downregulated in the urine of children with atopic dermatitis as compared to healthy controls ($p=0.05$) and has recently been reported to serve as a biomarker for the severity of inflammation. A receiver operating characteristic (ROC) curve analysis was performed and the area under the curve (AUC) for this miRNA was 0.6821.[69]

Obstetrics

In 2008, Chim et al.,[70] first, investigated the role of miRNAs as a potential biomarker for pathologic pregnancy.[70] Later, it has been demonstrated that circulating trophoblast-derived miRNAs reflected the physiological status of the pregnancy and could be used diagnostically.[71] A miRNAs urine profile has been explored in pregnant women with intrahepatic cholestasis (ICP) in order to identify a potential biomarker. Comparing the ICP patients and the healthy controls, 24 miRNAs presented significantly different expression levels. Among them, 15 miRNAs were upregulated ($p<0.05$) and 9 were downregulated ($p<0.05$) in the ICP group.[72]

Neurology

Despite the recent interesting findings of circulatory miRNAs in the neurologic setting, such as in Alzheimer's disease,[73] multiple sclerosis,[74] and Parkinson's disease,[75] the evaluation of these markers in the urine or in the cephalorachidian fluid is still in a primordial phase.

Liver

The use of circulating miRNAs as biomarkers has been assessed in liver disorders, such as drug-induced liver injury,[76] chronic viral hepatitis,[77,78] HCC,[79] and non-alcohol-related fatty liver disease.[80] Conversely, the investigation of urinary miRNAs in liver disorders has been performed only in the oncologic field.

Urinary miRNAs in solid tumors

Few studies have been conducted mainly in the urologic setting to detect the presence of urinary miRNAs in different types of cancers.[81] During pathological processes like malignant diseases, the RNA turnover is faster than normal, which results in higher nucleosides' levels in the blood and urine. The modified nucleosides do not undergo the same processes as in normal conditions and are usually excreted intact into the urine.

It has been noticed that upregulated or downregulated levels of specific urinary miRNA are significantly higher in people affected by non-gynecologic (Table 1) and gynecologic (Table 2) solid tumors, compared to healthy controls. In particular, while the assessment of a single nucleoside might result in poor predictability, association of a set of miRNAs from urine samples in addition to traditionally adopted cancer biomarkers appears to increase both the sensitivity and specificity in detecting cancer at an early stage.

Deregulation of miRNAs has been first noticed in HCC. A study that was conducted in high-risk–hepatitis C patients in Egypt demonstrated that despite the poor predictive values of the findings, the sensitivity of miR-650 and the specificity of the mir-618/miR-650 combination were greatly improved compared to the alpha-fetoprotein (AFP)-level-based detection method (sensitivity of 68% and specificity of 75%). The proposed HCC miRNA signatures may be of great value for the early diagnosis of HCC before the onset of the disease among high-risk hepatitis C virus (HCV)-infected patients.[89] Similarly, with the aim to assess the diagnostic value of urine miRNAs in bladder cancer, a recent meta-analysis has documented that a combination of miRNAs, in blood and urine, may represent non-invasive biomarkers for an early diagnosis of bladder cancer.[106]

MiRNAs in ovarian cancer

Circulating miRNAs

The most commonly and widely used biomarkers in OC are serum CA-125 and HE4. However, as per the screening tool they both are unsatisfying in terms of sensitivity and specificity, even when their use is integrated with imaging screening methods and clinical evaluation.

MiRNAs have been investigated in OC given their proven alteration in other solid tumors. Indeed, OC is characterized by a wide-scale deregulation of miRNAs that has resulted mostly in downregulation through both the genetic and epigenetic mechanisms as shown by Zhang et al.[107] Shahab et al.[108] identified 33 overexpressed and 9 underexpressed miRNAs that differentiate OC from the normal ovarian surface epithelium. In particular, miRNAs from the miR-200 family were underexpressed in the normal human ovarian surface epithelium and overexpressed in OC ($p<0.05$).

Different expression of miRNAs has been investigated in different histological types of OC. Wyman et al.[109] found a set of 124 differentially expressed miRNAs in cancer samples as compared to healthy controls, and 38 miRNAs were differentially expressed across histologic subtypes of OC. Calura et al.[110] performed a study on 257 snap-frozen stage I epithelial OC biopsies that led to the identification

Table 1. Urinary miRNAs in solid tumors.

Authors	Tumor	Study groups	Upregulation	Downregulation	Sensitivity (%)	Specificity (%)
Hanke et al. (2010)[82]	Bladder	P: 18 C: 18	miR-126 miR-182	–	72	82
Yamada et al. (2011)[83]	Urothelial	P: 100 C: 44	miR-96 miR-183	–	71 74	89.2 77.3
Ahumada-Tamayo et al. (2011)[84]	Prostate	P: 9 C: 9	miR-196b mir-5743p miR-7c miR-7d miR-7e miR-7g miR-200b miR-149 miR-20b miR-17 miR-184 miR-20a miR-106a	miR-150 miR-328	NR	NR
Haj-Ahmad et al. (2014)[85]	Prostate	P: 8 B: 22	miR-1825	miR-484	45	75
Miah et al. (2012)[86]	Bladder	P: 68 C: 53	miR-15b miR-135b miR-1224-3p other miRNAs	–	94.1 NS	51 NS
Snowdon et al. (2012)[87]	Bladder	P: 8 C: 5	miR-126 miR-125b	–	80	100
Wang et al. (2012)[66]	Bladder	P: 51 C: 24	miR-155	miR-192 miR-200 family miR-192	NR 100 NS	NR 52.6 NS
Yun et al. (2012)[56]	Bladder	P: 207 C: 144	–	miR-145 miR-200	77.8 84.1	61.1 61.1
Von Brandenstein et al. (2012)[88]	Kidney	P: 23 C: 5	miR-15a	–	NR	NR
Abdalla et al. (2012)[89]	Liver	P: 106 C: 12	miR-625 miR-532 miR-618	miR-516-5p miR-650	58	75
Bryant et al. (2012)[90]	Prostate	P: 118 C: 17	miR-107 miR-574-3p	–	67	43
Kim et al. (2013)[91]	Bladder	P: 138 C: 144	miR-214	–	NR	NR
Megual et al. (2013)[92]	Bladder	P: 181 C: 136	miR-187 miR-18a miR-25 miR-92a	miR-142-3p miR-140-5p miR-204 miR-125b	84.8 NS NS 84.9	86.5 NS NS 74.1
Tolle et al. (2013)[93]	Bladder	P: 36 C: 19	miR-155b-5p miR-618	–	85 70	68.4 68.4
Srivastava et al. (2013)[94]	Prostate	P: 36 C: 12	–	miR-205 mir-214	89	80
Zhou et al. (2014)[95]	Bladder	P: 112 C: 78	miR-106b	–	76.8	72.4
Zang (2014)[96]	Bladder	P: 6 C: 3	–	miR-99a miR-125b	86.7 81.4	81.1 87
Sapre et al. 2014)[97]	Prostate	P: 16 P/C: 17	miR-16 miR-201 miR-222	–	NR	NR
Korzeiniewski et al. (2015)[98]	Prostate	P: 71 C: 18	miR-483-5p	–	NR	NR

(Continued)

Table 1. *(Continued)*

Authors	Tumor	Study groups	Upregulation	Downregulation	Sensitivity (%)	Specificity (%)
Stephan et al. (2015)[99]	Prostate	P: 38 C: 38	miR-183 miR-205	–	NR	NR
Debernardi et al. (2015)[100]	Pancreas	P: 46 C: 55	miR-143 miR-30-e	–	83.3	96.2
Yun et al. (2015)[101]	Prostate	P: 99 B: 51	hsv1-miR-H18 hsv2-miR-H9-5p	–	NR	NR
Eissa et al. (2015)[102]	Bladder	P: 188 C: 170	miR-210 miR-10b miR-29c	–	71.3 80.9 71.3	91.1 91.1 88.9
Wang et al. (2015)[103]	Bladder	P: 372 C: 69	miR-214	–	90.5	65.6
Salido-Guadarrama et al. (2016)[104]	Prostate	P: 73 C: 70	miR-100/200b	–	NR	NR
Sasaki et al. (2016)[105]	Bladder	P: 28 C: 19	miR-146a-5p miR-301b miR-563	–	100% NR NR	53.3% NR NR

MiRNA: microRNA; P: cancer; C: control group (benign disease and/or healthy people); NR: not reported; NS: not significant; P/C: control group: low risk.

Table 2. Urinary miRNAs in gynecologic malignancies.

Authors	Tumor	Study groups	Upregulation	Downregulation	Sensitivity (%)	Specificity (%)
Erbes et al.[52]	Breast	P: 24 C: 24	miR-155	miR-21 miR-125b miR-451	83.3	87.5
Záveský et al.[54]	Ovarian and endometrial	P: 16 C: 13	miR-92a miR-200b	miR-106b miR-100	NR	NR
Zhou et al.[53]	Ovarian	P: 39 C: 50	miR-30a-5p	37 different miRNAs	NR	NR

MiRNA: microRNA; P: cancer; C: control group (benign disease and/or healthy people); NR: not reported.

of robust miRNA markers for clear cell and mucinous histotypes. The clear cell histotype is characterized by a five-fold higher expression of miR-30a and miR-30a, whereas the mucinous histotype has five-fold higher levels of miR-192/194.[110] In another study, 18 miRNAs distinguished clear cell carcinoma from high-grade serous carcinoma. Among these, miR-509-3-5p, miR-509-5p, miR-509-3p, miR-508-5p, and miR-510 were strong differentiators; high miR-200c-3p expression was associated with poor progression-free survival (PFS; $p=0.031$) and overall survival (OS; $p=0.026$) in patients with high-grade serous carcinoma.[111]

Taylor et al. demonstrated that microRNA profiles (miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, and miR-214) of ovarian tumors compared to those of tumor exosomes isolated from the same patients were similar (correlations from 0.71 to 0.90). Whereas exosomes miRNAs were detectable also in patients with benign ovarian disease as well (although significantly distincted from profiles observed in OC), they have not been detected in healthy controls.[112]

Later, the interplay of circulating miRNA expression in cancer has also been investigated with other molecules, such as Vitamin D.[113] The progressive enrichment in existing information related to OC biology has convincingly revealed that Vitamin D may induce expression of miRNAs, thus mediating inhibitory effects on cell proliferation.[114]

The identification of the miRNAs that significantly affect the tumor marker profile was a process that occurred gradually. Following, we reported the principal results.

Through qRT-PCR, Resnick et al.[115] compared the serum of OC patients with that of healthy controls and found miR-21, miR-29a, miR-92, miR-93, and miR-126 to be significantly overexpressed ($p<0.01$), whereas miR-99b, miR-127, and miR-155 levels were underexpressed in the former group ($p<0.01$).

In 2010, 24 blood samples from patients suffering from relapsed OC were evaluated and compared with blood samples of 15 normal subjects; expression levels of four miRNAs were significantly different between the two groups with miR-30c1 being upregulated in OC patients

and miR-342-3p, miR-181a, and miR-450b-5p being downregulated ($p < 0.05$).[116] Based on the miRNA profile, the discrimination between blood samples of OC patients and healthy controls has been estimated to reach an accuracy of >76%. When only serous subtypes were considered and compared with the extended group, the accuracy, the specificity, and the sensitivity increased to >85%.[116]

Interestingly, Zheng et al.,[117] in 2013, in a larger sample analysis (360 epithelial OC patients and 200 healthy controls), observed that the plasma levels of miR-205 were significantly higher and those of let-7f in samples from OC patients than controls; combination of these two miRNAs with serum CA 125 additionally improved the accuracy of the detection. Similarly, serum HE4 and miR-21 have shown a positive correlation ($r = 0.663$, $p < 0.0001$).[118] Furthermore, in the same study, a significant positive correlation between the relative expression levels of miR-21 (tumor/adjacent tumor tissue) and tumor grade has been found ($r = 0.608$, $p < 0.0001$), with an expression of miR-21 in tumor grade IV lesions which is significantly higher than that in tumor grade II–III lesions ($p = 0.0002$).[118] This finding suggests that miR-21 may be involved in the invasion and metastasis of tumor cells, and it may be a marker for poor prognosis of OC.

MiR-200a, miR-200b, and miR-200c were significantly elevated in the serum of 28 patients affected by serous epithelial OC when compared to controls ($p < 0.05$) even in the study led by Kan et al.[50] A multivariate model combining miR-200b and miR-200c gave the best predictive power to discriminate serum from OC patients and healthy subjects, suggesting that the evaluation of a set of miRNAs rather than a single one could improve the sensitivity and specificity.[50]

In 2014, Shapira et al., compared control plasma with pre-surgical plasma from patients with OC, found that 19 miRNAs were underexpressed and 3 overexpressed in patients with cancer. However, only six of them—miR-106b, miR-126, miR-150, miR-17, miR-20a, and miR-92a—were able to distinguish between plasma from cancer patients and healthy control. Significant difference was found in the expression of five miRNAs in women with short and long overall survival (miR-720, miR-20a, miR-223, miR-126_3p, and miR-1290 were highly expressed in women with short overall survival (<2 years) compared to women with longer overall survival; $p < 0.05$).[119]

Interestingly, the assessment of circulating miRNAs could be useful not only in the early detection of the disease but also as a biomarker of the response to treatment and drug resistance. Benson et al.[120] investigated the expression levels of miRNAs in patients before and after chemotherapy. Of 13 miRNAs, 10 (miR-193a-5p, miR-375, miR-339-3p, miR-340-5p, miR-532-3p, miR-133a-3p, miR-25-3p, miR-10a-5p, miR-616-5p, and miR-148b-5p) displayed changes in the concentration ranging from −2.9- to 4-fold ($p < 0.05$) in recurrent platinum-resistant OC patients, and concentrations of miR-148b-5p was correlated with the PFS ($p < 0.05$). Zhu et al. proposed that miRNA expression patterns may play an important role in drug resistance among OC. They investigated the relationship between resistance to paclitaxel and miRNA expression showing that expression of the miR-134 gene cluster is significantly lower in the paclitaxel-resistant cell line than in the paclitaxel-sensitive cell line, while the expression of the miR-17-92 gene cluster is significantly higher in the paclitaxel-resistant cells. An analysis of miRNA target–gene protein expression also revealed that several targets of miR-17-92 are significantly altered between the two cell types. These findings suggested that the higher expression of miR-17-92 and lower expression of miR-134 and the associated alterations of the target gene expression may be associated with the drug-resistant nature of some OCs.[121]

Recently, the Multicenter Italian Trials in Ovarian Cancer (MITO)-group identified 35 miRNAs that predicted risk of progression or relapse in OC patients and used them to create a prognostic model, the 35-miR-based predictor of Risk of Ovarian Cancer Relapse or progression (MiROvaR). It allows classifying patients into a high-risk group (89 patients with a median PFS of 18 months (95% confidence interval (CI): 15–22)) and a low-risk group (90 patients with a PFS of 38 months (24–not estimable), hazard ratio (HR): 1.85, 95% CI: 1.29–2.64, $p = 0.00082$). MiROvaR represents a significant predictor of progression in the two validation sets (OC263—HR: 3.16, 95% CI: 2.33–4.29, $p < 0.0001$ and OC452—HR: 1.39, 95% CI: 1.11–1.74, $p = 0.0047$) and maintains its independent prognostic effect when adjusted for relevant clinical covariates using multivariable analyses (OC179—adjusted HR: 1.48, 95% CI: 1.03–2.13, $p = 0.036$; OC263—adjusted HR: 3.09, 95% CI: 2.24–4.28, $p < 0.0001$; and OC452—HR: 1.41, 95% CI: (1.11–1.79), $p = 0.0047$).[122]

Urinary miRNAs

In OC, two important studies have investigated the role of urinary miRNAs.[53,54] Záveský et al.[54] examined the expression of cell free urine miRNAs in OC and endometrial cancer patients. They enrolled patients with epithelial OC, fallopian tube cancer, endometrial cancer, and benign diagnosis undergoing gynecological surgery secondary to the suspected diagnosis of ovarian and endometrial cancers. They compared the expression between pre- and post-surgery OC samples, and they aim to find out whether cell-free miRNAs may be detected and differentially expressed in urine of patients particularly with OC and endometrial cancers as compared to control patients. In total, 18 miRNAs were tested. The results showed that four miRNAs (miR-92a, miR-200b, miR-106b, and miR-100) were significantly differentially expressed between OC and control samples. The miR-92a and miR-200b were

upregulated, and miR-106b along with miR-100 was downregulated in cancer samples as compared to control samples. The limitation of this study consisted in the reduced number of overall tested samples.

Another attempt to investigate the expression on miRNAs in the urine of OC patients was made by Zhou et al.,[53] who collected and compared urine samples from 39 ovarian serous adenocarcinoma patients, 26 patients with benign gynecological disease, and 30 healthy controls in order to determine the clinical value of urinary mRNAs in the detection of ovarian serous adenocarcinoma. The results were promising: the miRNAs microarray data showed that only miR-30a-5p was upregulated and 37 miRNAs were downregulated in the urine samples of ovarian serous adenocarcinoma patients when compared to healthy controls. The upregulation of urinary miR-30a-5p was closely associated with early stage ovarian serous adenocarcinoma and with metastatic disease to the lymph nodes. Furthermore, urinary miR-30a-5p from OC patients was notably reduced following the surgical removal of the cancer, suggesting that urinary miR-30a-5p was derived from the ovarian serous adenocarcinoma tissue. The same pattern was not observed in other solid tumors such as gastric cancer and colon–rectal carcinoma patients suggesting that the upregulation of urinary miR-30a-5p may be specific for ovarian serous adenocarcinoma.[53]

Despite these interesting and promising findings, further studies need to be carried out in larger scales to better assess the significance and the role of urinary miRNAs in OC patients.

MiRNAs in breast cancer

Circulating miRNAs

In BC, the data show a potential role of deregulated miRNAs as modulators of carcinogenesis, proliferation, apoptosis, and drug-resistance.[123–125] Their presence in serum and plasma suggest that they could represent as potential novel biomarkers for early detection and outcome prediction. Nine miRNAs are actually relevant in discriminating BC from healthy controls or as predictors in therapy response (miR-21, miR-34a, miR-125b, miR-155, miR-195, miR-200b, miR-200c, miR-375, and miR-451).[52] High expression of circulating miR-34a and miR-155 in serum was associated with primary metastatic BC ($p<0.05$) and high miR-34a levels correlated with an advanced stage of disease ($p=0.01$).[126] Furthermore, a significant correlation between serum miR-122 and miR-375 levels and neoadjuvant chemotherapy response in locally advanced BC has been documented.[127]

Upregulation of miR-125b serum levels in BC patients significantly discriminates BC patients from healthy controls, and it is able to predict chemotherapeutic resistance.[49,128] MiRNAs may have a potential role in the therapeutic setting as well: the capability of miR-200 family in blocking tumor angiogenesis by the inhibition of the epithelial–mesenchymal transition may represent a potential relevant therapeutic strategy and a predictive parameter in BC therapy.[129]

Finally, the expression of circulating miRNAs in BC has also been correlated with the presence of circulating tumor cells (CTCs). Higher expression levels of miR-200b and miR-200c were observed in serum from CTC-positive metastatic BC patients compared to CTC-negative patients, suggesting them as indicators for CTC-status and as a prognostic marker in metastatic BC.[128]

In the recurrent setting, seven miRNAs were found to be differentially expressed in BC patients with and without recurrences. Four miRNAs were upregulated (miR-21-5p, miR-375, miR-205-5p, and miR-194-5p) and three miRNAs were downregulated (miR-382-5p, miR-376c-3p, and miR-411-5p).[130]

Urinary miRNAs

Before miRNAs were detected in the urine of patients with BC, numerous studies had already shown alteration of the urinary nucleoside concentration in these patients.

However, based on the results achieved in the other malignancies, the interest has been restricted from the urinary nucleosides to the miRNAs, secondary to the poor specificity of the urinary nucleosides in detecting and differentiating cancer.

The only available study published so far has evaluated the differences found in the expression of four BC-associated miRNAs quantified as median miRNA expression levels.[52] Urinary miR-155 levels were significantly higher in BC patients as compared to healthy controls (1.49 vs 0.25, $p<0.001$). In contrast, as compared to healthy controls, BC patients exhibited significantly lower urinary levels of miR-21 (2.27 vs 5.07, $p<0.001$), miR-125b (0.71 vs 1.62, $p<0.001$), and miR-451 (0.02 vs 0.59, $p=0.004$). Higher sensibility and specificity appear to be associated with the evaluation of a set of urinary miRNAs rather than a single one and through the integration of serum levels of traditionally used tumor biomarkers.

MiRNAs and BRCA mutations

Recent studies have demonstrated a relationship between BRCA mutations (BRCAm) and miRNAs, particularly BRCA1. BRCA1 regulates the expression of miRNAs, which may in turn regulate the expression of BRCA1.[131,132]

Seven miRNAS targeting BRCA1 with upstream signal have been identified, these include miR-182,[133] miR-146a,[134] miR-146-5p,[135] miR-15a, miR-16,[136] miR-638,[137] and miR-17;[138] in addition, six miRNAs targeted by BRCA1 with upstream signal have been identified, these include miR-155,[60,61] miR-148, miR-152,[139] miR-205,[140] miR-99b, and miR-146a.[141,142] This list of BRCA regulated

miRNAs suggests that loss of BRCA1 can upregulate oncogenic miRNAs or downregulate tumor-suppressive miRNAs, and it opens a new scenario that may lead to the development of new targeted therapies. Furthermore, because miRNAs often act as downstream effectors of protein kinases or driver genes mutated in cancer, targeting miRNAs may represent a strategy to increase specificity and overcome drug resistance.

The use of miRNA agents, such as an upregulating oncogenic miRNA antagomirs or downregulating tumor-suppressive miRNAs mimic, in BRCA1-associated cancer, for instance, would be of great interest. Furthermore, the combination of these miRNA agents with other therapeutic drugs might be a useful strategy for treating BRCA1-associated human cancers, including BC and OC.

As of now, the differentiation between BRCAm and wild type cancer patients based on urinary miRNAs has not been investigated yet. We are currently investigating this possibility in OC patients.

Conclusion

Since the identification of circulating miRNAs in OC and BC patients, and of the correlation with clinical data and prognosis, attempts have been made to identify miRNAs in other biological fluids. Urine seems to be a potential source of biomarker in several diseases, including solid tumors. Although preliminary, identification of different specific urinary miRNAs in OC and BC is giving promising results in diagnostic setting. Furthermore, because miRNAs act as key molecule downstream of oncogenic pathways involved in cancer progression, it provides the rationale for their use as also promising target for therapy.

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